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Optogenetics: Applications in neurobiology

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Abstract

Commonly used neuromodulation techniques such as electrical stimulation or pharmacologic intervention have some technical limitations that preclude dissecting particular cell- or pathway-specific functions in the brain, which is composed of billions of neurons. An advancement of molecular genetics techniques has provided a novel method in neuroscience called optogenetics. Optogenetics uses a combination of genetic and optical methods that provide a means to, with great temporal precision, experimentally control the activation or suppression of specific neuronal sub-populations in heterogeneous brain regions where multiple neuronal subtypes exist; this approach can be performed even on freely moving animals. Thus, this tool can uniquely assist in establishing causality between the disorder and the underlying pathology. Ongoing exploration of pathological mechanisms in various animal models of neuropsychiatric disorders with precise tools such as optogenetics can provide significant advances in the development of more focused approaches to treatment of these disorders. Here, we selectively highlight the major advancements gained by the use of optogenetic tools to uncover at circuit levels mechanisms relevant to neuropsychiatric disorders.

Keywords: optogenetics, opsin, neuropsychiatric disorders, addiction, schizophrenia, stress, depression, Parkinson's disease, Alzheimer's disease.

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Introduction

The brain is composed of billions of neurons which form uncountable connections, with each circuit having multiple cell types, firing properties, various signaling events, and wiring patterns. Pathways of various compositions communicate in such a way that they not only control neighboring networks, but they are themselves controlled by surrounding circuits. Multiple neurotransmitters released with various patterns to activate postsynaptic receptors on other neurons are simultaneously involved in this complicated interaction. Since brain tissue has a heterogeneous nature, it is very difficult to define the role of specific neuronal types in intact circuits. However, for complete understanding of the pathology of brain disorders, it is necessary to identify the underlying particular neural circuits using precise and specific methods and approaches. Despite the progress made in the last decades using various neuromodulation techniques (i.e. electrical stimulation or pharmacologic intervention), many questions remain regarding the exact circuitries and neurochemical mechanisms involved in certain pathological conditions. Indeed, it has been incredibly difficult to completely discern the underlying molecular mechanisms with these traditional tools, which are not able to dissect cell-type or pathway-specific function of a behavioral response. In fact, previous approaches either simultaneously affected multiple types of cells and processes in the targeted area, or had slow kinetics and reversibility.

Advancement of molecular genetics has created revolutionary improvements in approaches studying normal and pathological brain functioning. Since Karl Deisseroth and colleagues developed optogenetics via microbial opsin engineering by combining genetic methods and optical instruments for guiding light to activate or inhibit the specific neural circuits to manipulate behavior with temporal precision, it has been widely applied in fundamental research of neural circuits involved in different kinds of behaviors and pathogenic mechanisms of brain diseases (Boyden et al., 2005; Tye and Deisseroth, 2012). The recent emergence of these tools provides a new effective approach for establishing the causal relationships between selective neuronal activity and behavior. Optogenetics uses a combination of genetic and optical methods to control the activity of specific cells of living tissue even within freely moving animals. This provides a means to experimentally control the activation or inhibition of specific neurons in heterogeneous brain regions where multiple neuronal sub-populations exist, and to do so with precise temporal resolution (Deisseroth et al., 2006). Here, we will discuss the principal strengths of optogenetic manipulations to uncover at circuit levels the mechanisms relevant to certain neuropsychiatric disorders.

Optogenetic tools

Multiple components are required for a successful optogenetic study (Deisseroth, 2015). This technology most commonly involves three core features: microbial opsins (Berndt et al., 2011; Gradinaru et al., 2010; Mattis et al., 2011; Nagel et al., 2002, 2003; Zhang et al., 2007, 2011), specific opsin gene expression which targets well-defined cellular elements in the brain (Atasoy, Aponte, Su, and Sternson, 2008; Gradinaru et al., 2010; Kuhlman et al., 2008; Tsai et al., 2009), and neurochemical release in targeted brain regions elicited by certain wavelengths of light (Adamantidis et al., 2007; Aravanis et al., 2007; Bernstein et al., 2008; Diester et al., 2011; Grossman et al., 2010; Iwai et al., 2011; Schneider, Gradinaru, Zhang, and Deisseroth, 2008; Yizhar et al., 2011a).

Opsins, light-sensitive proteins, are a crucial tool for optogenetics. By inserting different exogenous light-activated ion channels and pumps, neural circuits can be activated or inhibited in response to light depending upon the ion selectivity of the protein. Currently, the most widely applied opsin is a codon optimized form of Channelrhodopsin-2, ChR2(H134R). ChR2 is a blue-light-activated cation channel from the single cell green alga *Chlamydomonas reinhardtii* (Nagel et al., 2003). When ChR2 is expressed in neurons, its activation by blue light opens the channel, thereby depolarizing the membrane, which allows sodium and potassium ions to flow down their electrochemical gradient into the cell. This blue light-activated cation channel allows millisecond-precision excitation of neurons (Nagel et al. 2005; Towne and Thompson, 2016). Conversely, there is a microbial opsin that has been optimized to push the membrane voltage in the opposite direction and thus inhibit neural activity (Han et al., 2011; Stefaniket et al., 2013). Halorhodopsin, from the archaebacterium *Natronomonas pharaonis* (NpHR), is a yellow light-activated protein that pumps negative chloride ions into the cell, hyperpolarizing neural membranes and inhibiting activity (Zhang et al., 2007). Archaerhodopsin-3, from the archaebacterium *Halorubrum sodomense* (Arch), is a green light-activated protein that pumps protons out of the cell, also hyperpolarizing neural membranes and inhibiting activity (Chow et al., 2010). Together these three proteins comprise the basis of the majority of optogenetic studies reported in the literature.

The optogenetic opsin protein family is rapidly expanding. Newly found opsins are sensitive to higher wavelengths: yellow/red-shifted ChR types such as channelrhodopsin-1 from *Volvox carteri* (VChR1), a red-shifted channelrhodopsin (ReaChR), and Chrimson are some of the recent ones (Klapoetke et al., 2014; Lin et al., 2013; Zhang et al., 2008). These wavelengths are more suitable because they have less absorption and scattering in the brain tissue; therefore, they allow for the optical activation of particularly deep neurons (Lin et al., 2013).

Currently, genetic engineering methods enable the expression of these opsins in specific cell types and desired subcellular locations. While the tools for achieving genetic specificity to opsin delivery are constantly improving, there are currently three general methods to transport opsins to neurons in vivo. The use of transgenic mice, which stably express an opsin under the control of a specific promoter, is one of the common methods (Liske et al., 2013). The limitations of this method are the lack of spatial specificity of opsin expression and the time and effort required to generate new mouse lines for new opsins and promoters. Another approach is the use of viral vectors with neural tropism, such as lentivirus and adeno-associated virus (AAV) (Fenno, Yizhar, and Deisseroth, 2011). These vectors can be engineered to express an opsin under the control of a specific promoter, then injected into the brain region of interest, where they can be light-activated from a fiber optic cable guided to the area of infusion via a surgical implant. An advantage of this approach is that transgenic animals are not required, allowing delivery into species that are not easily amenable to transgenesis, such as rats and primates. The disadvantage of using vectors is that AAV is relatively small with a limited packaging capability, often cited as 4.7–5.4 kilobases (Grieger and Samulski, 2005), and cannot be used in targeting strategies that require large cell-type-specific promoters (Fitzsimons, Bland, and During, 2002). The final approach integrates the ad-

vantages of transgenic mice with the brevity and precision targeting of viral methods. A large variety of mouse lines has been generated that expresses Cre-recombinase under different cell-type-specific promoters. This recombinase recognizes loxP sites in DNA that flank a gene and can flip the sequence into the correct orientation to allow transcription (or vice versa). When a virus expressing an inverted and floxed (flanked by loxP sites) opsin is introduced into a Cre-recombinase expressing cell, the enzyme inverts the opsin into the correct orientation to allow gene expression. Adjacent cells that do not express the Cre-recombinase are infected by the virus but do not express the opsin. By combining this with projection-based strategies, highly specific circuit targeting can be achieved (Towne and Thompson, 2016).

Optogenetic applications

The innovative application of opsin proteins for optogenetics has revolutionized many fields of biomedical research and particularly neuroscience. Therefore, it is not surprising that *Nature Methods* considered this approach the "Method of the Year" in 2010. The application of optogenetic methods has allowed us a more sophisticated understanding of neuronal circuits underlying complex behaviors, such as those involved in psychiatric disorders.

Schizophrenia

Cortical inhibitory interneuron dysfunction may play an important role in the pathophysiology of certain psychiatric disorders, including autism, schizophrenia and various cognitive disorders that are associated with social deficits (Benes, 2010; Marin, 2012; Rubenstein and Merzenich, 2003; Uhlhaas and Singer, 2010; Yizhar et al., 2011b; Yizhar, 2012). Schizophrenia symptoms include hallucinations, delusions, disorganized speech and catatonic behavior, as well as negative symptoms such as emotional flatness, apathy and lack of speech (Endicott and Spitzer, 1978; Kay, Fszbein, and Opler, 1987).

With its complex cytoarchitecton structure, the neocortex contains multiple neuronal subtypes, including distinct classes of inhibitory interneurons and excitatory pyramidal neurons (Freund, 2003; Isaacson and Scanziani, 2011; Markram et al., 2004). Optogenetics allows for a clearly defined way to distinguish between these functionally discrete cell types and to record and manipulate neural activity in a precise way, unlike electrical stimulation. The breakdown in the transmission or processing of neural information is one symptom of schizophrenia, which is thought to be correlated to an imbalance between excitation and inhibition, therefore altering gamma oscillations, which are considered to have a potential role in the transfer of information between brain regions (Kehrer, Maziashvili, Dugladze, and Gloveli, 2008; Lewis, Hashimoto, and Volk, 2005). A specific class of GABA interneurons that express the calcium-binding protein parvalbumin (PV) and have fast-spiking (FS) electrophysiological properties is widely believed to generate cortical gamma oscillations (Freund T., 2003; Fuchs et al., 2007; Sohal, 2012; Tamas, Buhl, Lorincz, and Somogyi, 2000; Whittington, Traub, and Jefferys, 1995; Ylinen et al., 1995). Traditional neurobiological methods made it difficult to selectively manipulate PV neurons; however, optogenetic tools allow us to control PV neuron activity on a millisecond time scale.

Two recent studies used optogenetics to directly test the role of PV neurons. Cardin et al. showed that optically-induced activation of PV interneurons at 40–50 Hz is sufficient to induce gamma frequency local field potential (LFP) oscillations, while activation of pyramidal neurons enhanced only lower frequency oscillations and did not elicit LFP gamma oscillations in the barrel cortex of PV-Cre mice (Cardin et al., 2009). Gamma frequency modulation of excitatory input was also found to heighten cortical circuit performance by reducing circuit noise (Cardin et al., 2009; Sohal et al., 2009). Additionally, this study showed that gamma frequency LFP oscillations generated by PV interneurons can gate sensory inputs, demonstrating a potential role for interneuron-driven oscillations in cortical processing. Next, using an array of optogenetic technologies (Aravanis et al., 2007; Boyden et al., 2005; Zhang et al., 2007), Deisseroth and his group have demonstrated that inhibition of PV interneurons via halorhodopsin (eNpHR) suppresses gamma oscillations, whereas activation of these neurons drives gamma oscillations in vivo (Sohal et al., 2009). These two studies have clearly revealed the ability of PV neurons to generate cortical gamma rhythms and their role in information processing in the neocortex, which may help to understand information processing deficits in schizophrenia (Cardin et al., 2009; Sohal et al., 2009).

Severe behavioral deficits in psychiatric diseases such as schizophrenia have been hypothesized to arise from a disruption in the homeostatic balance between cortical excitation and inhibition (E/I balance), possibly reflecting mechanisms such as impairments in PV interneurons within neural microcircuitry (Kehrer, Maziashvili, Dugladze, and Gloveli, 2008; Markram et al., 2010; Rubenstein and Merzenich, 2003; Rubenstein et al., 2010; Vattikuti et al., 2010). Yizhar et al. tested this hypothesis in freely moving mice using several optogenetic tools. This study found that increasing activity in excitatory neurons within the medial prefrontal cortex disrupted the social behavior of mice, whereas optogenetically elevating activity within prefrontal interneurons did not. Thus, elevation, but not reduction, of cellular E/I balance elicits a profound impairment in cellular

information processing associated with specific behavioral impairments (Yizhar et al., 2011b).

Addiction

Optogenetics has importantly contributed to investigation of the neuronal pathways related to reward seeking, and has allowed identification of the adaptations that take place in these circuits following chronic exposure to drugs of abuse (Cao, Burdakov, and Sarnyai, 2011; Lobo, 2012; Stuber, Britt, and Bonci, 2012).

Two highly interconnected brain regions play critical roles in mediating reward: the ventral tegmental area (VTA) and the nucleus accumbens (NAc). The VTA is a heterogeneous brain structure that contains different neuronal populations, which include dopamine (DA), GABA and glutamate neurons. DA neurons in the VTA are the main effectors of reward (Berke and Hyman, 2000; Grace, 2000; Gonzales, Job, and Doyon, 2004; Stuber, Britt, and Bonci, 2012; Weiss and Porrino, 2002). It is well known that DA can be released naturally with two main patterns: phasic and tonic (Grace, 1991; Grace, 2000; Schultz, 1998; Wightman and Robinson, 2002). Tonic DA firing occurs at a low frequency of 5–10 Hz and results in steady-state DA concentrations that are lower than 50 nM (Justice, 1993; Parsons and Justice, 1992). In contrast, phasic, or burst, firing of DA neurons at frequencies of more than 30 Hz leads to large, transient increases in DA concentrations, which may significantly exceed 50 nM (Aragona et al., 2008; Freeman, Meltzer, and Bunney, 1985; Grace and Bunney, 1983, 1984; Hyland et al., 2002; Wightman and Robinson, 2002; Wightman and Zimmerman, 1990). These distinct characteristics of phasic and tonic DA release suggest divergent roles of each pattern in the control of DA-related behaviors. Recently, the emergence of optogenetics has allowed us to better explore the causal relationship between accumbal DA and addictive behavior. Optogenetic activation of VTA DA neurons can selectively induce DA release in accumbal terminal fields with very high temporal and spatial precision. Moreover, optical stimulation of the VTA was shown to mimic phasic and tonic DA release (Bass et al., 2013; Tsai et al., 2009). Using a combinatorial viral-mediated gene delivery approach to express ChR2 in mesolimbic DA neurons in rats, our group revealed that tonic optogenetic stimulation of VTA-nucleus accumbens DA release significantly decreased reward consummatory behavior, possibly by masking phasic activity that is thought to be essential for reward-based behaviors (Bass et al., 2013; Mikhailova et al., 2016). Using the same approach, Tsai and his group showed that phasic, but not tonic, DA release is solely responsible for the development of conditional place preference (CPP) (Tsai et al., 2009).

These results demonstrate that phasic DA activity is sufficient to mediate behavioral conditioning (Tsai et al., 2009). Furthermore, phasic activation of DA neurons causally enhances positive reinforcing actions in a food-seeking task (Adamantidis et al., 2011). Moreover, phasic activation was sufficient to reactivate previously extinguished food-seeking behavior in the absence of external cues (Adamantidis et al., 2011) and to enhance the initiation of approach behavior without long-term motivational regulation (Ilango et al., 2014). Optogenetic activation of DA neurons, mimicking a prediction error, was sufficient to cause long-lasting increases in cue-elicited, reward-seeking behavior (Steinberg et al., 2013).

The notion that changes in excitatory drive onto NAc neurons is central to drug effects is further strengthened by studies that have directly manipulated the activity of D1 dopamine receptor (D1R) or D2 dopamine receptor (D2R) containing MSNs (Bock et al., 2013; Lobo et al., 2010) or the cholinergic interneuron populations within the NAc (Witten et al., 2010). Lobo et al. used optogenetic tools to selectively control the firing rate of D1R and D2R neurons in the NAc and studied consequent effects on cocaine reward. This study showed that D1R and D2R containing neurons in the NAc have distinctly different effects on cocaine conditioned place preference: activating D1R-expressing neurons enhances cocaine CPP, whereas activating D2R-expressing neurons suppresses cocaine CPP (Lobo et al., 2010). These results provide insight into the molecular control of D1R and D2R neuronal activity as well as the circuit level contribution of these cell types to cocaine reward (Lobo et al., 2010). Optogenetic methods helped to establish the role of cholinergic cells, despite the fact that they represent less than 1% of NAc neurons (Witten et al., 2010). In related work that also combined optogenetics with the use of CPP, it has been shown that light-induced silencing of cholinergic neurons in the NAc blocked cocaine conditioning in freely moving mammals.

Stuber et al. demonstrated that optical stimulation of the excitatory projections from the BLA to the NAc, but not cortical projections, in mice produced robust self-stimulation. Moreover, this self-stimulation of BLA-NAc projections was dependent on D1R signalling. Brief optical inhibition of BLA-to-NAc fibers reduced cue-evoked intake of sucrose, demonstrating an important role of this specific pathway in controlling naturally occurring reward-related behavior. These data suggest that while the BLA is important for processing both positive and negative effects, the BLA-to-NAc glutamate pathway in conjunction with DA signaling in the NAc promotes motivated behavioral responding (Stuber, Britt, and Bonci, 2011). Optogenetic self-stimulation of DA neurons in the VTA has also been demonstrated (Adamantidis et al., 2011; Witten et al., 2011). Cohen et al. showed that activation of VTA GABA neurons inhibits VTA DA neurons in vivo and counteracts excitatory drive from

primary reward when the reward is expected. In addition, these neurons were excited by aversive stimuli, potentially contributing to suppression of firing in some DA neurons in response to aversive events (Cohen et al., 2012). Research is also underway to understand why drug-seeking behaviors persist despite severe adverse consequences, which is a hallmark of addiction (Chen et al., 2013; Seif et al., 2013). Chen et al. indicated that cocaine self-administration decreases ex vivo intrinsic excitability of deep layer pyramidal neurons in the prelimbic cortex, which was significantly more pronounced in compulsive drug-seeking animals. Furthermore, compensating for hypoactive prelimbic cortex neurons with in vivo optogenetic prelimbic cortex stimulation significantly prevented compulsive cocaine seeking, whereas optogenetic prelimbic cortex inhibition significantly increased compulsive cocaine seeking (Chen et al., 2013). Together, these studies clearly demonstrate how optogenetics has revealed the causality of changes in brain neurotransmission for addictive behaviors and has begun to identify related circuitries.

Stress and Anxiety

Commonly comorbid with addiction, stress-related disorders can influence reward seeking and reward taking behavior and have been similarly explored using optogenetics. Anxiety disorders are a result of aberrant stress responses characterized by a hyper-alert state of arousal which can be exhibited as a multitude of varied symptoms (Lieb, 2005). Due to the diverse manifestations of anxiety disorders, understanding the underlying circuitry driving these stress-related behaviors is an important step towards effectively treating them.

A group of regions that has garnered close examination in stress and anxiety disorders includes the shell region of the NAc, the bed nucleus of the stria terminalis (BNST), and the medial (MeA), central (CeA), and basolateral (BLA) nuclei of the amygdala (sometimes refered to as the "extended amygdala"; Sparta, Jennings, Ung, and Stuber, 2013). The BNST, in particular, has been the focus of numerous optogenetic studies (Garcia-Garcia et al., 2017; Partridge et al., 2016; Xu et al., 2016) due to its connectivity to both stress regions, particularly the amygdala, and regions involved in reward, such as the mesolimbic pathway (Sparta Jennings, Ung, and Stuber, 2013). For example, it was shown that modulation of BNST GABA inputs can occur when optically stimulated in the presence of an agonist for kappa opioid receptors, which play a role in stress-induced behavioral changes (Li et al., 2012). While some studies show that stress and stress-induced signaling can lead to changes in neuronal activity, other regions studied have demonstrated that neuronal activation can induce stress and anxiety-like behavior. It was recently found that optical activation of β-adrenergic receptor signaling via Gαs in BLA excitatory neurons was able to induce anxiety-like behaviors in mice (Siuda et al., 2016). These experiments show that the application of optogenetics can enable scientists to explore the effects of stressful stimuli on neuronal function but also the effects of neuronal function on behavior displays, whether in the presence of a stressful stimuli or not.

Other regions and pathways have also been examined in stress and anxiety disorders, including structures with connections to the amygdala such as the medial prefrontal cortex (mPFC), locus coeruleus (LC), and hippocampus (Felix-Ortiz et al., 2016; Luthi and Luscher, 2014; McCall et al., 2015; Seo et al, 2016; Vialou et al., 2014), as well as other circuitry such as the septohypothalamic circuit (Anthony et al., 2015). The LC is of particular interest because of its prominent role in the stress response. It was found that optically-induced corticotropin releasing hormone (CRH) release from projections from the amygdala to the LC resulted in anxiogenic behavior (McCall et al., 2015). Alternatively, optical stimulation of LC noradrenergic terminals in the BLA also induced anxiety-like behavior (McCall et al., 2017). Interestingly, studies have been able to take advantage of the specificity of optogenetics to target distinct efferent and afferent projections between the two regions. For example, optical stimulation of projections from the mPFC to the BLA inhibited cholecystokinin-induced anxiety (Vialou et al., 2014), while optical stimulation of inputs from the BLA to the mPFC was also anxiogenic (Felix-Ortiz et al., 2016). This is important because the ability to isolate projections and induce precise activation (or inhibition) can help establish causality in connecting the circuit of activity within these regions of interest with the related stress and anxiety-like behavior.

Depression

Depression is a common mental disorder characterized by persistent sadness and a loss of interest in activities (Kessler et al., 2003). The major behavioral symptoms include anhedonia and deficits in several aspects of reward. This suggests a certain degree of overlap among the brain regions affected by depression and drug addiction (Russo et al., 2013).

One experiment elicited a depression-related phenotype (increased social avoidance and decreased sucrose preference) in mice undergoing a subthreshold social defeat paradigm with optogenetic phasic activation of VTA DA neurons (Chaudhury et al., 2013). Activation of these neurons also quickly induced a similar phenotype in previously resilient mice that had been subjected to repeated social defeat stress (Chaudhury et al., 2013). Furthermore, the differences in projection pathway-specificity in promoting stress susceptibility

were shown: optical activation of NAc-projecting or inhibition of PFC-projecting VTA DA neurons induced susceptibility to social-defeat stress (Chaudhury et al., 2013). In contrast to these results, following a chronic mild stress protocol, which is different from the acute social defeat stress, optogenetic phasic activation of VTA DA neurons acutely rescued the chronic mild stress-induced depression-like phenotype and alleviated deficits in sucrose preference assays while selective inhibition of VTA DA neurons acutely produced depression-related behavior in freely moving rodents (Tye et al., 2012).

Two other key brain areas of depression-related circuitry that have been targeted using optogenetics are the mPFC and dorsal raphe (DR). The mPFC contains different neuronal populations, and it is believed that this structure plays an important role in various neuropsychiatric diseases, including depression. However, an understanding of the mechanisms by which the mPFC neurons are involved in this disorder has remained elusive. A series of optogenetic studies were carried out to investigate the role of different classes of mPFC neurons in depression-related behavior. Covington et al. (2010) performed the first optogenetic study of the mPFC in a mouse model of depression and showed that optogenetic activation of the mPFC induced an antidepressant-like effect in susceptible mice following chronic social defeat stress. Activation of mPFC terminals in the brainstem DR nucleus increased motor activity during the forced swim test, but did not affect general locomotor activity in the open field test, suggesting that this projection is particularly important for responding to behavioral challenges (Warden et al., 2012). Further work revealed that activation of mPFC terminals in the NAc generated antidepressant-like effects following social defeat stress, whereas mPFC terminal activation in the BLA had anxiolytic effects (Vialou et al., 2014).

Parkinson's disease

Parkinson's disease (PD), a common neurodegenerative disease, is characterized by rigidity, tremor, postural instability and slow movement (Gelb, Oliver, and Gilman, 1999; Hughes, Daniel, Kilford, and Lees, 1992). PD results from the death of dopamine neurons in the substantia nigra (SN). Pharmacological approaches to elevate DA levels and deep brain stimulation (DBS) have been the conventional treatments for PD for many decades. Pharmacological interventions, however, have various limitations and side effects following long-term use. Although DBS in the subgenual cingulate cortex has been used in humans to treat depression (Mayberg et al., 2005), DBS in the subthalamic nucleus (STN), a part of the basal ganglia circuit, and in other targets, has shown some therapeutic effects for treatment of the motor mainfestations of Parkinson's disease (Benabid, 2003; Deuschl et al., 2006; Kumar et al., 1998). However, the precise cellular mechanisms of this therapy have been unclear and highly controversial. Because of the heterogeneity of brain tissue where electrodes are placed, it has been challenging to elucidate the major target cell types involved or the underlying mechanisms of DBS.

In 2009, it was reported that therapeutic effects could be accounted for by direct and selective stimulation of afferent axons projecting into the STN using optogenetics and solid-state optics to drive or inhibit an array of distinct circuit elements in freely moving parkinsonian rodents (Gradinaru et al., 2009). High-frequency stimulation of afferent fibers projecting from the motor cortex to the STN ameliorated motor symptoms, while optogenetic excitation and inhibition of subthalamic nucleus neurons had no effect. Thus, these results demonstrated an optical approach for dissection of disease circuitry and provided a new way for systematic deconstruction of disease circuits by selectively controlling individual components (Gradinaru et al., 2009). These data suggested a model for DBS treatment in which white matter tracts or axonal pathways are the most effective direct target of control. These data were later confirmed by other studies (Li et al., 2012).

Optogenetics has also been used to probe the mechanisms underlying the efficacy of grafting human pluripotent stem cell-derived DA neurons in disease models. Animals with striatal 6-OHDA lesions exhibit motor impairments that are strongly reversed following engraftment of DA neurons in the lesioned area (Kriks et al., 2011). To probe the mechanisms underlying the efficacy of this effect, Steinbeck et al. optogenetically inhibited engrafted DA stem cells after motor recovery. They reported that inhibiting DA neurons resulted in the reappearance of motor deficits, indicating the essential role of DA neurons at the lesion site (Steinbeck et al., 2015).

Alzheimer's disease

Alzheimer's disease (AD) is the debilitating neurodegenerative disorder most commonly observed in the elderly, in which patients have problems with language, disorientation, and impaired declarative memory that affects neuronal activity at many levels. AD is the most common form of dementia that results from damage of neurons and local circuits in specific brain regions (Palop et al., 2006). The aggregation of amyloid β (Aβ) peptides is considered one of the hallmarks of this pathological dysfunction in the brains of AD patients (Selkoe et al., 2012). Studies using electrical or pharmacological stimulations have shown that certain patterns of neuronal activity can modulate the formation and secretion of Aβ plaques from neurons (Cirrito et al., 2005, 2008; Kamenetz et al., 2003), however, the exact mechanism of this phenom-

enon remains largely unknown. Optogenetics was used to selectively activate a specific neuronal pathway in APP transgenic mice in order to observe the causative role between a certain type of synaptic activation and Aβ pathology (Yamamoto et al., 2015). Their findings suggest a connection between overactivity of the specific projection pathway and augmentation in Aβ deposition. This study also provided the foundation for further research using optogenetics for chronic stimulation in animal models of neurodegenerative disorders.

Long-term memory is essential for cognition, and its disruption is pivotal to multiple neurolpsychiatric disorders, especially AD. Bero et al. found that optogenetic excitation of mPFC neurons inhibits the activation of entorhinal-hippocampal circuits and impairs the encoding of long-term associative memory (Bero et al., 2014). Since the development of optogenetics, there have also been some new discoveries about the mechanism of memory retrieval. It was found that contextual fear memory recall could be abolished by optogenetic inhibition of the hippocampus, but could not be influenced by pharmacological inhibition. It indicated that long-term memory retrieval, which is dependent on the hippocampus, could shift to other structures like the anterior cingulate cortex (Goshen et al., 2011; Rajasethupathy et al., 2015).

Conclusion

The use of optogenetics has greatly improved the precision with which we are able to examine discrete neuronal subpopulations in a cell- and time-specificic manner unattainable through conventional methods. Furthermore, this tool can uniquely be used to establish causality between the disorder and the underlying biology. As we examine different neuropsychiatric disorders with precise tools such as optogenetics, the accumulated data will guide the future development of new therapeutic approaches that will become more focused.

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